Stromal Acidosis Modulates Corneal Swelling

Nancy A. McNamara, Kenneth A. Polse, and Joseph A. Bonanno

Purpose. Studies have shown that stromal acidosis reduces the rate of corneal thickness recovery after induced edema, providing the first human in vivo evidence that corneal pH can influence corneal hydration control. This finding raises the question of the possible effect that pH may have on induced corneal swelling. To explore this question, the corneal swelling response to hypoxia was measured while stromal pH was controlled.

Methods. Corneal edema and stromal acidosis was induced in ten subjects by passing a mixture of nitrogen and carbon dioxide gas across the eyes through tight-fitting goggles. One eye of each subject received 100% N₂, whereas the contralateral eye received a mixture of 95% N₂ and 5% CO₂. Exposures of 95% N₂ + 5% CO₂ lower pH on average to 7.16 versus 7.34 for 100% N₂ alone. Before and after 2.5 hours of gas exposure, central corneal thickness (CCT) was measured.

Results. Eyes exposed to the lower pH environment (eg, N₂ + CO₂) developed less change in CCT compared to the eyes receiving N₂ alone. Overall increase in CCT was 29.9 ± 5.3 μm for eyes exposed to the 95% N₂ + 5% CO₂ gas mixture, versus 37.1 ± 4.8 μm for 100% N₂ eyes (P < 0.0001).

Conclusions. The corneal swelling response to hypoxia can be reduced by lowering stromal pH. Because changes in corneal pH alone have not been found to alter steady-state CCT, it is proposed that pH exerts its effect only under non-steady-state conditions (ie, corneal swelling and deswelling). This suggests that acidosis may produce changes in the rate of lactate metabolism or alter endothelial hydraulic conductivity.


Overall corneal hydration control can be assessed by measuring the rate of corneal deswelling after inducing corneal edema. It has been shown that if the eye is continuously exposed to a low-pH environment while the cornea recovers from induced edema, the rate of thickness recovery is slower compared to the rate at which the cornea deswells under normal pH conditions. To our knowledge, this result provided the first human in vivo evidence that corneal pH can influence corneal hydration control. This finding raises the question of how stromal acidosis might affect the corneal swelling response during hypoxic conditions, because reduced oxygen tension at the corneal epithelium causes increased hydration and stromal acidosis.

A hypothesis that pH might influence hypoxic corneal swelling seems tenable because there is considerable evidence that suggests that pH has substantial effects on cell physiology. For example, it has been shown that reduced pH can cause corneal endothelial cell edema and junctional breakdown, decrease endothelial Na⁺/K⁺-ATPase activity, reduce net sodium flux, diminish transendothelial potential and fluid flux, and reduce epithelial Cl⁻ transport. Also, when human stromal pH is lowered by exposing the tears to carbon dioxide, transient morphologic changes have been observed in the corneal endothelium. These pH-mediated effects on cell physiology and corneal deswelling rate have prompted us to speculate that pH might also influence corneal swelling. Earlier work has shown that exposure to a CO₂-induced low-pH environment has no effect on steady-state corneal thickness; however, these previous studies have not specifically examined the influence of pH on corneal swelling under non-steady-state conditions (ie, exposure to hypoxia). In the current study, we explore...
the effects of stromal acidosis on corneal hydration by inducing edema via hypoxia while the stromal pH is lowered by exposing the eye to a humidified CO₂ environment.

MATERIALS AND METHODS

Subjects

Ten subjects, five men and five women (mean age, 23.9 years, range, 21 to 29 years), were recruited from the campus of the University of California, Berkeley. All subjects were free of external ocular disease. Three of the ten subjects wore hydrogel lenses on a daily basis, and were instructed to discontinue lens wear a minimum of 12 hours before the taking of measurements. Before the initial testing, subjects received a full explanation of the procedures and informed consent was obtained. The tenets of the Declaration of Helsinki were followed, and approval was granted by the Committee for Protection of Human Subjects.

Corneal Thickness

Corneal thickness was measured using a modified Haag-Streit (Bern, Switzerland) optical pachometer equipped with fixation lights for improved measurement accuracy. The pachometer potentiometer was linked to an IBM-compatible microcomputer for providing direct entry of data to the computer memory, which allowed accurate time monitoring and facilitated data collection and analysis. This instrument has been more fully described elsewhere.11

Hypoxic and pH-Controlled Corneal Environments

To explore the effects of different pH levels on corneal swelling, humidified, temperature-controlled 5% ± 0.1% CO₂ gas balanced with nitrogen was passed across the cornea using air-tight swim goggles. Previous studies have used the same experimental conditions to induce stromal acidosis, and have shown that exposure to pure N₂ gas alone produces a stromal pH of 7.34, whereas a 95% N₂ + 5% CO₂ gas exposure reduces pH to approximately 7.16; both of these pH values are below that which is found under open-eye conditions (7.54 ± 0.01).12

Procedures

To monitor the corneal swelling response under different pH conditions, one eye each of ten subjects was exposed to 100% N₂ gas, and the contralateral eye received a mixture of 95% N₂ + 5% CO₂. All ten participants were awake at least 2 hours before corneal thickness readings were measured. The procedure consisted of first estimating the steady-state central corneal thickness (e.g., baseline thickness) by taking four sets of pachometry measurements separated by approximately 15 minutes. Each set of pachometry readings consisted of ten replicate readings that were averaged to provide one set of thickness readings. Each of the four sets were then averaged to provide the value for the steady-state corneal thickness. The mean baseline corneal thickness of the group was 498.5 ± 26.7 μm (range, 441 to 542 μm). A comparison of the mean baseline of the three contact lens wearers to the non-lens wearers was 513 versus 492 μm, respectively, with thickness values for both groups within normal limits.13 This slight difference in corneal thickness may have represented residual swelling secondary to daily contact lens wear, and therefore the data analysis was done both using the entire group and without the three lens wearers.

After estimating the baseline corneal thickness, a standard pair of air-tight swim goggles, which were modified to allow for input and flow of the gases, was fitted to each subject to provide a local gas environment for each eye. Edema was induced by passing temperature-controlled (37°C), humidified 100% N₂ gas across the cornea of one eye, while the contralateral eye received the humidified gas mixture of 95% N₂ and 5% CO₂. The gas environment of either N₂ or the N₂ + CO₂ mixture was arbitrarily assigned to the subject’s right or left eye, and the eye selected for either of these gas environments was done without knowledge of either the subject or observer (double masked). The goggles were left in place for approximately 2.5 hours.

After exposure to the gas-controlled environments, the right goggle was removed (the left goggle was held in place), and two sets of central corneal thickness measurements were taken of the right eye. After these readings, the left goggle was removed and pachometry measurements taken. A third and fourth set of thickness measurements were taken on both eyes within 15 minutes of the first set. For each eye, these two sets of measurements were averaged and compared to baseline pachometry to determine the amount of corneal swelling that occurred over the 2.5 hours of gas exposure.

RESULTS

Table 1 summarizes the results, including baseline corneal thickness values, change in corneal thickness, eye receiving the N₂ + CO₂ gas environment, and the average swelling values for each group. Before exposure to the anoxic environment, the mean baseline central corneal thickness of the ten subjects corresponded to 499 ± 27.2 μm and 498 ± 29.1 μm for the right and left eyes, respectively. No significant difference in thickness between the right and left corneas was found (P = 0.88). After exposure to the experi-
TABLE 1. Corneal Swelling Response for Paired Eyes Following 95% N₂ + 5% CO₂ Versus 100% N₂ Exposure

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Pregas Thickness (µm)</th>
<th>N₂ + CO₂ (OD/OE)</th>
<th>Change in Corneal Thickness (µm)</th>
<th>% Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD</td>
<td>OS</td>
<td>N₂</td>
<td>N₂ + CO₂</td>
</tr>
<tr>
<td>1</td>
<td>453</td>
<td>441</td>
<td>OD</td>
<td>.30</td>
</tr>
<tr>
<td>2</td>
<td>508</td>
<td>494</td>
<td>OD</td>
<td>.29</td>
</tr>
<tr>
<td>3</td>
<td>516</td>
<td>519</td>
<td>OS</td>
<td>.31</td>
</tr>
<tr>
<td>4</td>
<td>499</td>
<td>499</td>
<td>OS</td>
<td>.39</td>
</tr>
<tr>
<td>5</td>
<td>520</td>
<td>524</td>
<td>OS</td>
<td>.25</td>
</tr>
<tr>
<td>6</td>
<td>466</td>
<td>472</td>
<td>OS</td>
<td>.28</td>
</tr>
<tr>
<td>7</td>
<td>513</td>
<td>511</td>
<td>OD</td>
<td>.23</td>
</tr>
<tr>
<td>8</td>
<td>542</td>
<td>538</td>
<td>OD</td>
<td>.34</td>
</tr>
<tr>
<td>9</td>
<td>475</td>
<td>471</td>
<td>OD</td>
<td>.35</td>
</tr>
<tr>
<td>10</td>
<td>502</td>
<td>507</td>
<td>OS</td>
<td>.25</td>
</tr>
<tr>
<td>Mean</td>
<td>499.4</td>
<td>497.6</td>
<td>OD = 4</td>
<td>37.1</td>
</tr>
<tr>
<td>SD</td>
<td>27.2</td>
<td>29.1</td>
<td>OS = 6</td>
<td>4.8</td>
</tr>
</tbody>
</table>

P < 0.0001  P < 0.0001

mental environment, there was substantial increase in corneal hydration in both eyes; however, for each subject, the eye that received the additional CO₂ exposure (lower pH) consistently developed less swelling. This reduced swelling response during exposure to the 95% N₂ + 5% CO₂ environment occurred for all ten subjects, and was not substantially different in the three contact lens wearers. The mean increase in central corneal thickness for those ten eyes that received the 100% N₂ gas was 37.1 ± 4.82 µm (7.64% ± 1.37%), compared to 29.9 ± 5.00 µm (6.28% ± 1.16%) for the companion eyes, which received the 95% N₂ + 5% CO₂ gas mixture. It is unlikely that these differences in corneal swelling under the two experimental conditions are due to chance alone (paired t test, n = 10, P < 0.0001), and any residual effects of contact lens wear that may have been present did not appear to effect significantly the results under the paired-eye study design (paired t test, n = 7 non-contact lens wearers, P < 0.001).

Figure 1 illustrates the swelling responses of right and left eyes that were exposed to either the 100% N₂ or 95% N₂ + 5% CO₂ environments. For comparison of the same subject, the right and left eyes are plotted with matching symbols and connected by a straight line. Each point represents the percentage increase in corneal hydration for one eye after exposure to either 100% N₂ or 95% N₂ + 5% CO₂ gas. Inspection of right and left eyes for each paired-eye comparison shows that the eyes receiving the 95% N₂ + 5% CO₂ gas consistently swelled less than the eyes receiving 100% N₂ gas.

DISCUSSION

This study provides evidence that stromal pH can modulate the degree of corneal swelling that occurs during exposure to an anoxic environment. The reduced anoxic swelling response in the presence of CO₂ is unlikely to be due to CO₂ alone because previous studies have shown that, under steady-state conditions, exposure of the cornea to 7% and 9% CO₂ at normal oxygen tensions does not alter corneal hydration. This
suggests that the reduced stromal pH resulting from the CO\textsubscript{2} exposure affects corneal hydration only under non-steady-state conditions (ie, during swelling or deswelling).

It is interesting that previous reports comparing the amount of corneal edema resulting from hypoxic contact lens wear to that caused by anoxic environment (eg, 100% N\textsubscript{2} gas without contact lens wear) have shown that contact lens-induced hypoxic swelling is greater than that caused by pure anoxia.\textsuperscript{10,14} This observation led us initially to hypothesize that the increased stromal acidosis that accompanies contact lens wear, as compared to pure anoxia, might account for these observations. Our findings, however, indicate that the greater swelling accompanying hypoxic contact lens wear cannot be explained by a pH-based mechanism, and therefore must be caused by some other factor (changes in epithelial permeability, increased temperature, and the like).

Our data show that the corneal swelling response to hypoxic stress can be reduced by lowering stromal pH. Earlier reports have demonstrated that acidosis causes a delay in recovery time after induced edema, compared to the recovery that occurs under normal pH conditions.\textsuperscript{2} This paradox, that both corneal swelling and deswelling are reduced during low stromal pH conditions, is not easily explained.

One possible explanation for this paradox may be that pH affects epithelial or endothelial metabolism during hypoxic conditions. For example, hypoxic corneal swelling is the result of an accumulation of lactate in the cornea secondary to stimulation of glycolytic activity.\textsuperscript{15,16} Because it has been shown that glycolytic activity is pH sensitive,\textsuperscript{17} it is possible that lowering stromal pH decreases lactate production during swelling and reduces lactate elimination during deswelling. It also is possible that stromal acidosis inhibits the active transport processes of the endothelium; however, this would likely increase the overall swelling response with osmotic movement of fluid into the cornea, which is contrary to our findings. Also, our findings would not support a pH-dependent decrease in endothelial function because earlier studies have shown that endothelial transport processes are not pH sensitive in the range that corresponds to our experimental conditions.\textsuperscript{18,19}

It also is possible that reduced pH decreases the stromal swelling pressure, leading to less imbibition of water and thus less swelling. In vitro studies have shown water uptake of dried corneal stroma to be constant in the range that human pH might vary,\textsuperscript{20} suggesting that the swelling pressure is not being altered by the changes in pH encountered in the current study. Moreover, if changes in pH altered stromal swelling pressure, then thickness (ie, hydration) would have been affected by CO\textsubscript{2} application under normal oxygen conditions, and this was not found in previous studies.\textsuperscript{21}

Finally, it may be that pH affects the corneal barrier function. Under steady-state conditions, specific ions are actively transported out of the cornea by the endothelium, while water passively follows. The state of corneal hydration depends, therefore, on both the concentration gradient across the endothelium and membrane permeability. Although the effect of stromal acidosis on the passive flow of fluid across the endothelium has not been previously studied, a change in corneal pH could decrease the conductivity of the endothelium. Because the hydraulic conductivity affects the rate of water moving in and out of the stroma, it would be expected that under steady-state conditions, when net flow of ions and water is near zero, a change in hydraulic conductivity would have no effect on net fluid flow. During corneal swelling or deswelling, however, a drop in hydraulic conductivity would slow the rate of influx and efflux, respectively, of water from the stroma, and could account for both the reduction in the rate of corneal swelling and recovery that occur under low-pH conditions.

This “hydraulic conductivity hypothesis” is consistent with some human in vivo studies in which it has been shown that acute exposure of the cornea to hypoxia causes reversible changes to the endothelium (ie, the endothelial bleb response).\textsuperscript{10} Other studies have demonstrated that chronic exposure to hypoxia can lead to permanent changes in endothelial morphology.\textsuperscript{21-25} These morphologic changes may subsequently reduce hydraulic conductivity across the endothelium and thereby increase the resistance to water flow.

In conclusion, stromal acidosis reduces the corneal swelling response to hypoxia and decreases the rate at which the cornea recovers from edema. Studies directed at understanding the underlying mechanism that produces this paradox may help further our understanding of those factors that regulate corneal hydration control. Suggested mechanisms may include a pH-induced decrease in the production and elimination of lactate during hypoxia, or possible alterations in hydraulic conductivity across the corneal endothelium.

**Key Words**

stromal acidosis, corneal function, corneal edema, hypoxia, endothelial function

**References**

2. Cohen S, Polse K, Brand R, Bonanno J. Stromal acido-